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Deciphering the Regulatory Network of MicroRNAs in Tuberculosis Infected Macrophages

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

In

Genetics

At Massey University, Albany,
New Zealand.

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2017

Abstract

Tuberculosis is an infectious disease that is caused by *Mycobacterium tuberculosis* (*Mtb*), an intracellular pathogen that uses macrophages as a host for replication. The outcome of the disease depends highly on *Mtb*'s strategies to circumvent the immune responses of macrophages. MicroRNAs (miRNAs) are small regulatory RNAs that influence gene functions post-transcriptionally. Recent studies indicate that miRNAs have prominent roles in cellular host-pathogen interactions. The aim of this study is to advance our understanding of the regulatory mechanisms that control key miRNAs in mouse M1 macrophages during *Mtb* infection using network analysis.

The study began with a construction of a mouse miRNA-centric regulatory network model by combining a network of miRNA-controlling transcription factors (TFs) with a miRNA target network. The final network places miRNAs at the center of a comprehensive regulatory network of TFs, miRNAs and their targets. This network represents a useful resource for investigating miRNA functions and their control. Subsequently, we populated the network with CAGE-derived expression data for either *Mtb*-infected mouse M1 macrophages or non-infected controls. We used network analysis to determine key regulatory elements during the infection process. As a result, we identified a core set of TFs and miRNAs, which are likely critical regulatory elements during M1 macrophage host and *Mtb* interactions. Our results also demonstrate that among the core set of regulatory elements three highly activated miRNAs, mmu-mir-149, mmu-mir-449a, and mmu-mir-449b, work in unison with mmu-mir-155, the top-ranked miRNA. They co-regulate a set of downstream tuberculosis immune response related genes. Four top-ranked TFs, Fos11, Bhlhe40, Egr1, and Egr2, were identified that they transcriptionally control this group of miRNAs. The TFs and miRNAs, together with their targets constitute a mmu-mir-155 regulatory sub-network. Our results also imply that

Bhlhe40 is likely an important TF that modulates the activities of the mmu-mir-155 regulatory sub-network. Bhlhe40 and the mmu-mir-155 regulatory sub-network may be exploited by *Mtb* to manipulate the host immune defense for advancing survival interests. The findings of this study provide new insights into the host immune regulatory mechanisms of activated macrophages that are essential to control tuberculosis.

Acknowledgements

This study took me two and half years to complete. I have learned so much during this process of research. I would like to offer my special thanks to my supervisor Dr. Sebastian Schmeier. He gave me many invaluable supports and advice with great patience and careful instructions. Without his guidance and help, I could not be able to finish this study. I also like to give thanks to my colleague Elena Denisenko for her support in resolving my PROMiRNA retraining problems. Her help is deeply appreciated.

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List of abbreviations

3'	Three prime
5'	Five prime
BCG	Mycobacterium Bovis
bp	Base-pair
CAGE	Cap analysis gene expression
ChIP-seq	Chromatin Immunoprecipitation sequencing
chr	Chromosome
DNA	Deoxyribonucleic acid
EMB	Ethambutol
FDR	False discovery rate
HIV	Human immunodeficiency virus
IFN- γ	Interferon gamma
IL	Interleukin
INH	Isoniazid
LPS	Lipopolysaccharide
M1	Classically activated
M2	Alternatively activated
MDR-TB	Multiple drug resistant strains of Tuberculosis
miRNA	MicroRNA
<i>Mtb</i>	Mycobacterium tuberculosis
NCBI	National Center for Biotechnology Information
NGS	Next generation sequencing
NO	Nitric oxide
nt	Nucleotide

PCR	Polymerase chain reaction
poly-A	Polyadenylation
pre-miRNA	Precursor microRNA
pri-miRNA	Primary microRNA
qPCR	Quantitative polymerase chain reaction
RISC	RNA-induced silencing complex
RLE	Relative Log Expression
RMP	Rifampicin
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SM	Streptomycin
TB	Tuberculosis
TF	Transcription factor
TFBS	Transcription factor binding site
TGF- β	Transforming growth factor beta
Th2	T helper 2
TNF- α	Tumor necrosis factor alpha
TSS	Transcription start site
UCSC	The University of California, Santa Cruz
WHO	World Health Organization